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Characterization of the Archaeal Ribonuclease P Proteins from *Pyrococcus horikoshii* OT3

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Ribonuclease P (RNase P) is a ribonucleoprotein complex involved in the processing of the 5′-leader sequence of precursor tRNA (pre-tRNA). Our earlier study revealed that RNase P RNA (pRNA) and five proteins (*Pho*Pop5, *Pho*Rpp38, *Pho*Rpp21, *Pho*Rpp29, and *Pho*Rpp30) in the hyperthermophilic archaeon *Pyrococcus horikoshii* OT3 reconstituted RNase P activity that exhibits enzymatic properties like those of the authentic enzyme. In present study, we investigated involvement of the individual proteins in RNase P activity. Two particles (R-3Ps), in which pRNA was mixed with three proteins, *Pho*Pop5, *Pho*Rpp30, and *Pho*Rpp38 or *Pho*Pop5, *Pho*Rpp30, and *Pho*Rpp21 showed a detectable RNase P activity, and five reconstituted particles (R-4Ps) composed of pRNA and four proteins exhibited RNase P activity, albeit at reduced level compared to that of the reconstituted particle (R-5P) composed of pRNA and five proteins. Time-course analysis of the RNase P activities of R-4Ps indicated that the R-4Ps lacking *Pho*Pop5, *Pho*Rpp21, or *Pho*Rpp30 had virtually reduced activity, while omission of *Pho*Rpp29 or *Pho*Rpp38 had a slight effect on the activity. The results indicate that the proteins contribute to RNase P activity in order of *Pho*Pop5 > *Pho*Rpp30 > *Pho*Rpp21 >> *Pho*Rpp29 > *Pho*Rpp38. It was further found that R-4Ps showed a characteristic Mg^{2+} ion dependency approximately identical to that of R-5P. However, R-4Ps had optimum temperature of around at 55°C which is lower than 70°C for R-5P. Together, it is suggested that the *P. horikoshii* RNase P proteins are predominantly involved in optimization of the pRNA conformation, though they are individually dispensable for RNase P activity *in vitro*.

Key words: pre-tRNA, *Pyrococcus horokoshii*, ribonuclease P, ribonuclease P proteins.

Abbreviations: pre-tRNA, precursor tRNA; pRNA, ribonuclease P RNA; RNase P, ribonuclease P; R-nP, reconstituted particle composed of pRNA and n proteins.

Ribonuclease P (RNase P) is a ubiquitous endonuclease that processes the 5′ leader sequence of precursor tRNA (pre-tRNA) (*1*, *2*). Although functionality of RNase P remains similar from bacteria to humans, chemical composition and enzymatic property of this enzyme differ in various organisms (*3*). Eubacterial RNase P is composed of a catalytic RNA (pRNA) and a single protein subunit, and in the presence of a high concentration of Mg^{2+}, the eubacterial pRNA itself can hydrolyze pre-tRNA *in vitro* (*4*). In contrast, eukaryotic RNase Ps comprise a single RNA moiety and as many as 10 proteins, and the RNA component itself has no catalytic activity *in vitro* (*5*–*7*). Hence, the eukaryotic pRNA cooperatively functions with protein subunits in substrate recognition and/or catalysis. Recently, it was reported that human proteins Rpp21 and Rpp29, together with the RNA subunit H1 RNA, are sufficient to obtain weak cleavage of the 5′ leader sequence of pre-tRNA *in vitro* (*8*).

In an earlier study, we showed by reconstitution experiments that pRNA and four proteins *Pho*Pop5, *Pho*Rpp21, *Pho*Rpp29, and *Pho*Rpp30 are essential for the RNase P activity of the hyperthermophilic archaeon *Pyrococcus horikoshii* OT3 (*9*). The reconstituted particle, however, had a lower optimal temperature (around at 55°C) as compared with 70°C of the authentic RNase P from *P. horikoshii*. This result suggested that the authentic RNase P would have an additional component(s) which participates in elevation of the optimal temperature. Subsequently, a fifth protein *Pho*Rpp38 was found to be involved in elevating the optimum temperature of the reconstituted RNase P (*10*). Thus, the *P. horikoshii* pRNA and five proteins reconstituted RNase P activity that exhibits enzymatic properties like those of the authentic enzyme. These studies indicated that the *P. horikoshii* pRNA, like eukaryotic counterparts, cooperatively functions with five protein subunits in catalysis. It is therefore essential to examine how protein subunits in archaeal RNase Ps exert their functions in the RNase P activity. The study will aid in establishing structure and function relationships of archaeal as well as eukaryotic RNase Ps, and the resulting information would ultimately shed light on the transition from the proposed RNA world to the modern protein world.

To this end, we have been studying structures of individual proteins from *P. horikoshii* RNase P, and have determined crystal structures of five proteins: *Pho*Rpp21 (PDB

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ID 1X0T) (*11*), *Pho*Rpp29 (PDB ID 1V76) (*12*), *Pho*Rpp30 (PDB ID 1V77) (*13*), *Pho*Pop5 (PDB ID 2CZV) (*14*), and *Pho*Rpp38 (PDB ID 2CZW) (*10*), and the essential amino acid residues in *Pho*Rpp21 and *Pho*Rpp30 for the *P. horikoshii* RNase P activity were assigned by site-directed mutagenesis (*11*, *13*). Furthermore, *in vivo* interactions between five protein subunits of RNase P in *P. horikoshii* OT3 were examined by using yeast two-hybrid system (*15*). Completion of the protein structures, together with the two-hybrid experiment, proposed a model for a possible arrangement of the protein subunits in the *P. horikoshii* RNase P (*14*).

In this study, we reinvestigated the *in vitro* reconstitution of the *P. hoirikoshii* RNase P activity by incubating a mixture of proteins with pRNA, and found that five reconstituted particles (R-4Ps) composed of four proteins and pRNA had the RNase P activities, albeit at reduced level compared to that of the reconstituted particle (R-5P) composed of pRNA and five proteins. Then, we characterized the particles R-4Ps (a set of single protein omission particles) to assess the functional importance of the individual proteins by comparing their RNase P activities with that of R-5P. The result shows that the proteins contribute to RNase P activity in order of *Pho*Pop5 > *Pho*Rpp30 > *Pho*Rpp21 >> *Pho*Rpp29 > *Pho*Rpp38. Taking a spatial model of the proteins into consideration, the results suggest that a correct assembly of the individual proteins would be required for RNase P activity, and the resulting complex would stabilize an active conformation of pRNA.

MATERIALS AND METHODS

Materials—Five RNase P proteins (*Pho*Pop5, *Pho*Rpp21, *Pho*Rpp29, *Pho*Rpp30, and *Pho*Rpp38), pRNA, and pre-tRNA^{Tyr} in *P. horikoshii* were prepared, as described previously (*9*, *10*). All nucleotide sequence data were cited in the *P. horikoshii* OT3 genome database (http://www.bio.nite.go.jp). All other chemicals were of analytical grade for biochemical use.

Assay for RNase P activity—The *P. horikoshii* RNase P activity was reconstituted *in vitro*, as described previously (*9*) with some modifications. In brief, the *P. horikoshii* RNase P was reconstituted *in vitro* by incubating pRNA with each five RNase P protein in an equimolar quantity. RNase P assays were carried out at 45°C or 65°C for indicated times in 50 mM Tris-HCl (pH 7.6), 60 μg/ml tRNA (Sigma), 1 U/μl RNase inhibitor (Promega), 2 nM labeled pre-tRNA^{Tyr} (about 5×10^4 cpm), 50 mM magnesium chloride, 600 mM ammonium acetate, and 60 mM ammonium chloride, unless otherwise stated. In this analysis, the pre-tRNA^{Tyr} was prepared by the *in vitro* transcription in the presence of [α-^{32}P]UTP, as described previously (*9*). The reactions were stopped by phenol extraction, and the reaction products were separated on 15% polyacrylamide denaturing gels in TBE buffer (900 mM Tris-borate containing 10 mM EDTA) at 47 W for 1 h. Gels were exposed to an Imaging Plate and reaction products were visualized by a PhosphorImager, FLA-5000 (Fuji Film). The reaction products of R-5P by incubation with pre-tRNA^{Tyr} for 30 min were used as a control.

Characterization of the Reconstituted Particles—To examine optimal temperature and optimal concentration of Mg^{2+} ion of the reconstituted particles, the RNase P activity were measured in the same manner as those described above at 40–80°C and 10–50 mM, respectively, for 5 min (R-5P), 10 min (R-4P lacking *Pho*Rpp38), 1 h (R-4P lacking *Pho*Rpp29), or 13 h (R-4Ps lacking *Pho*Pop5, *Pho*Rpp21, or *Pho*Rpp30).

Nomenclature—The RNase P proteins from *P. horikoshii* OT3 were designated according to their homology to the corresponding human proteins and the prefix *Pho* is added to differentiate them from homologous proteins from other organisms. This new nomenclature replaces the old ones: Ph1481p, Ph1496p, Ph1601p, Ph1771p, and Ph1877p which were based on their gene ID numbers with *Pho*Pop5, *Pho*Rpp38, *Pho*Rpp21, *Pho*Rpp29, and *Pho*Rpp30, respectively.

RESULTS

In Vitro *Reconstitution*—In a foregoing study, we found that four proteins and pRNA from hyperthermophilic archaeon *P. horikoshii* OT3 reconstituted the RNase P activity with an optimal temperature around at 50°C (*9*), and the addition of a fifth protein *Pho*Rpp38 elevated optimal temperature for the activity from at 50°C to 70°C (*10*). In order to examine a functional involvement of the individual proteins to RNase P activity, pairwise mixings with pRNA were done, and the resulting mixtures were characterized with respect to RNase P activity, as described under "MATERIALS AND METHODS" (Fig. 1). Under the conditions used, the reconstituted particle (R-5P) composed of five proteins and pRNA efficiently cleaved 5′ leader sequence of pre-tRNA for 30 min, as shown in Fig. 1 (lane P). In this analysis, the reconstituted particles were tested for a longer incubation with the pre-tRNA^{Tyr} to detect some cleavage activity. The result showed that two particles (R-3Ps) composed of three proteins *Pho*Pop5, *Pho*Rpp30, and *Pho*Rpp38 with pRNA (lane 18) or *Pho*Pop5, *Pho*Rpp30, and *Pho*Rpp21 with pRNA (lane 20), albeit to a lesser activity, showed a detectable RNase P activity for 5 h under conditions used, as shown in Fig. 1A. Although faint bands could be observed in lanes 12 and 13, their mobility appeared to be slower than that of the mature tRNA. Hence, we judged that they were not tRNA produced by RNase P activity. It was therefore concluded that the three proteins (*Pho*Pop5, *Pho*Rpp30 and *Pho*Rpp38 or *Pho*Pop5, *Pho*Rpp30 and *Pho*Rpp21) and pRNA are sufficient for the removal of 5′ leader sequence from pre-tRNA. Next, addition of fourth proteins strongly stimulated RNase P activity of the reconstituted particles (Figs. 1A and 1B, lanes 26–30). The reconstituted particles (R-4Ps) lacking *Pho*Rpp29, *Pho*Rpp21, or *Pho*Rpp38 exhibited a significant cleavage activity for 5 h (Fig. 1A, lanes 27–29), albeit at reduced level compared to that of the reconstituted particle (R-5P) composed of pRNA and five proteins. In addition, the reconstituted particles (R-4Ps) lacking *Pho*Rpp30 or *Pho*Pop5 showed the RNase P activity after a longer incubation (12 h) (Fig. 1B, lanes 26 and 30). The result indicates that the *P. horikoshii* RNase P proteins are individually dispensable for RNase P activity *in vitro*, though all five proteins and pRNA are required to produce the reconstituted particle (R-5P) that exhibits catalytic properties like those of the authentic RNase P.

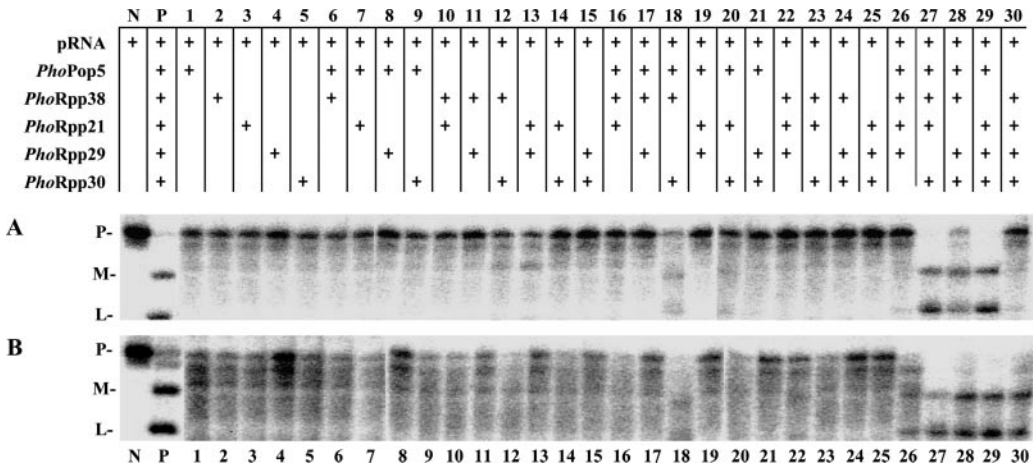

Fig. 1. ***In vitro* reconstitution of the *P. horikoshii* RNase P.** Combination of five proteins, as indicated by +, pRNA, and ^{32}P-labeled pre-tRNA^{Tyr} were diluted into 1× reaction buffer (50 mM Tris-HCl pH 7.6, 60 mg/ml tRNA, 1 U/μl RNase inhibitor, 50 mM magnesium chloride, 600 mM ammonium acetate, and 60 mM ammonium chloride). The cleavage reactions proceeded for 5 h (A) or 12 h (B) at 65°C. The reaction products were resolved on 15% acrylamide/8 M urea/TBE gels and visualized by autoradiography. Lanes N and P indicate the reaction products of pre-tRNA^{Tyr} digested with the *P. horikoshii* pRNA and R-5P, respectively. The lane P shows the reaction products of R-5P by incubation with pre-tRNA^{Tyr} for 30 min as a control. P, M, and L indicate the reaction products of pre-tRNA, mature tRNA and 5′-leader tRNA, respectively.

Involvement of the Individual Proteins in RNase P Activity—The present study showed that the *in vitro* reconstituted particles, R-4Ps, composed of four proteins and pRNA had the detectable RNase P activity. In order to evaluate a functional involvement of *P. horikoshii* RNase P proteins in RNase P activity, we analyzed a time-course (0–15 h) for the RNase P activities of R-4Ps and assessed contribution of the individual proteins to the RNase P activity (Fig. 2A). Figure 2, A and B, shows that the particles lacking *Pho*Rpp38 (c) or *Pho*Rpp29 (e) exhibited considerable activities, while those lacking *Pho*Pop5 (b), *Pho*Rpp21 (d), or *Pho*Rpp30 (f) had virtually reduced activity. In particular, the protein *Pho*Pop5 omission was found to have a great effect on the RNase P activity. Moreover, the incubation at a lower temperature (45°C) showed that the protein *Pho*Rpp38 made little contribution to the RNase P activity, suggesting that *Pho*Rpp38 would play a specific role in stabilization of pRNA at a higher temperature (Fig. 2C). The result indicates that the individual proteins contribute to RNase P activity in order of *Pho*Pop5 > *Pho*Rpp30 > *Pho*Rpp21 >> *Pho*Rpp29 > *Pho*Rpp38.

Characterization of R-4Ps—Next, RNase P activities of R-4Ps were characterized with respect to Mg^{2+} concentration and optimal temperature. Figure 3A showed that they had optimum activity around 30 mM Mg^{2+} concentration, which is similar to that of R-5P. The result suggests that the proteins are not involved in coordination of Mg^{2+} necessary for catalysis. Next, the optimum temperature for R-4Ps was compared with that for R-5P composed of five proteins and pRNA. As shown in Fig. 3B, the particles R-4Ps equally had an optimum temperature of around at 55°C (b–f), while with the R-5P particle it was around at 70°C (a). This finding suggests that pRNA interacted with five proteins folds in a more stable structure than that in R-4P and the five proteins are required to fold in an optimized conformation of pRNA at the higher temperature. Our preliminary experiment revealed that R-5P eluted faster than R-4P in gel filtration chromatography, suggesting that the pRNA in R-5P might fold in an oligomeric state distinct from that in R-4P (Hada and Kimura, unpublished results).

DISCUSSION

Since Altman and co-workers discovered that the *E. coli* RNase P RNA (M1 RNA) itself can hydrolyze pre-tRNA *in vitro* (*4*), biochemical and structural studies on RNase P have so far been mainly focused on eubacterial RNase Ps (for a review see Ref. *3*). These studies defined crucial nucleotides at helix P4 in RNase P RNA as sites for Mg^{2+} coordination important for catalysis (*16–19*) and identified a nucleotide as a recognition site for the 5′ leader sequence of pre-tRNA substrates (*20*). Recently, the crystal structures of RNase P RNAs from *Thermotoga maritima* (*21*) and *B. stearothermophilus* (*22*) were established. In addition, three-dimensional structures of eubacterial protein subunits (*23–25*) and roles of the protein subunit were extensively studied (*26*, *27*). Although a large number of information about structure and function relationships has become available for eubacterial RNase Ps, only a few studies have thus far been carried out with eukaryotic and archaeal RNase Ps.

In earlier study, we found that the four proteins (*Pho*Pop5, *Pho*Rpp21, *Pho*Rpp29, *Pho*Rpp30) and pRNA reconstituted RNase P activity, but the resulting particle had a lower optimal temperature (around at 55°C), as compared with 70°C of the authentic RNase P from *P. horikoshii* (*9*). Subsequently, it was found that addition of a fifth protein *Pho*Rpp38 to the reconstituted particle elevates the optimal temperature and also enhances RNase P activity (*10*). This finding led us to reinvestigate *in vitro* reconstitution of RNase P activity using five proteins and pRNA. The present result showed that the two particles (R-3Ps) composed of three proteins *Pho*Pop5, *Pho*Rpp30 and *Pho*Rpp21 or *Pho*Pop5, *Pho*Rpp30 and *Pho*Rpp38 exhibited a slight RNase P activity, suggesting that the three proteins (*Pho*Pop5, *Pho*Rpp30 and *Pho*Rpp21 or

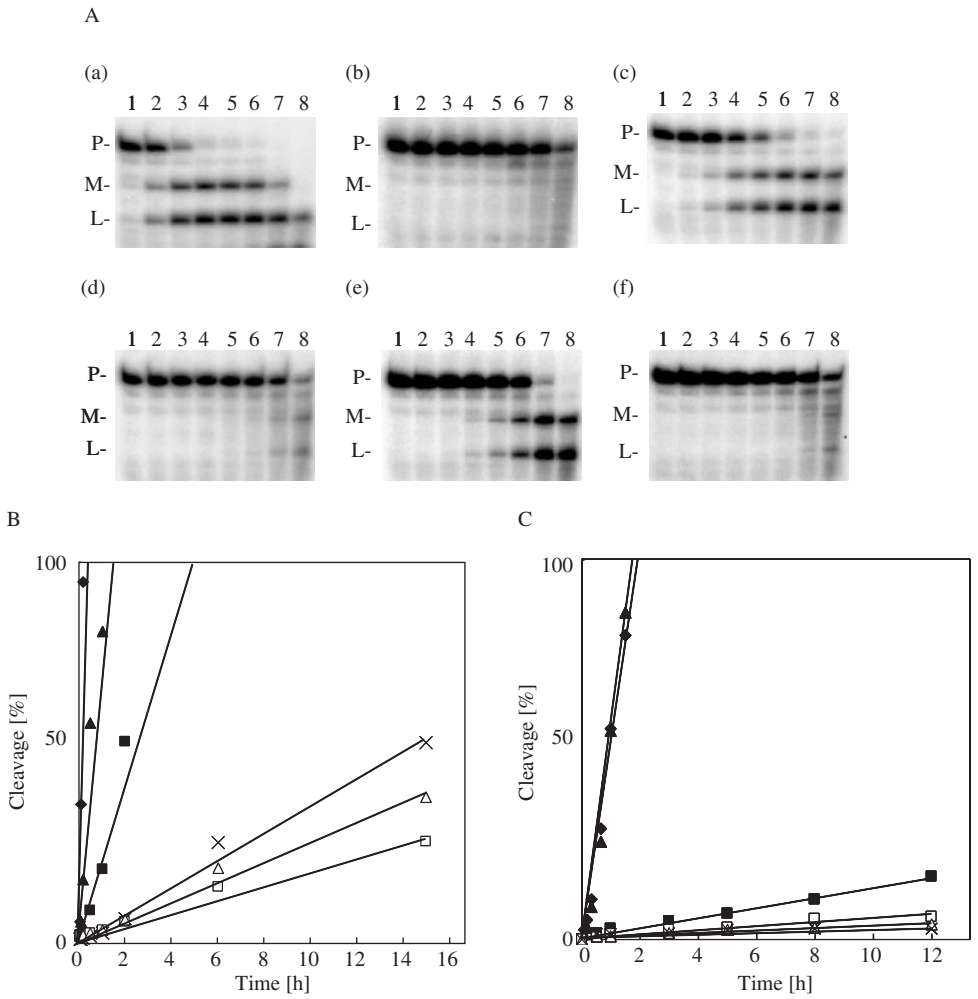

Fig. 2. **Time course analysis of RNase P RNA activity of R-4Ps.** A: Time course of cleavage of pre-tRNA^{Tyr} by the *P. horikoshii* pRNA reconstituted with four proteins (R-4Ps). Reaction volume was 20 μl and the cleavage reactions were performed at 65°C. Small aliquot of 10 μl each were withdrawn at the following time points: 2 min (lane 1), 5 min (lane 2), 10 min (lane 3), 30 min (lane 4), 1 h (lane 5), 2 h (Lane 6), 6 h (lane 7), and 15 h (lane 8), and the reactions were stopped by phenol extract. The cleavage products were resolved on 15% acrylamide/8 M urea/TBE gels and visualized by autoradiography. (a), R-5P; (b), R-4P lacking *Pho*Pop5; (c), R-4P lacking *Pho*Rpp38; (d), R-4P lacking *Pho*Rpp21; (e), R-4P lacking *Pho*Rpp29; (f), R-4P lacking *Pho*Rpp30. B: Graphic representation of the time course of substrate cleavage at 65°C seen in A. Solid diamonds, R-5P; open squares, R-4P lacking *Pho*Pop5; solid triangles, R-4P lacking *Pho*Rpp38; ×, R-4P lacking *Pho*Rpp21; solid sqaures, R-4P lacking *Pho*Rpp29; open triangles, R-4P lacking *Pho*Rpp30. C: Time course analyses were done at 45°C (data not shown) and the graphic representation is shown, as given in B.

*Pho*Rpp38) and pRNA are sufficient for pre-tRNA processing activity. This result is in disagreement with the previous result that assigned *Pho*Pop5, *Pho*Rpp21, *Pho*Rpp29, and pRNA as the minimal components of *P. horikoshii* RNase P (*9*). Although we have at present no appropriate explanation for discrepancy, we can not exclude the possibility that the reaction product produced by *Pho*Pop5, *Pho*Rpp21, *Pho*Rpp30, and pRNA was mistakenly analyzed as that produced by *Pho*Pop5, *Pho*Rpp21, *Pho*Rpp29, and pRNA in the previous experiment.

Five particles (R-4Ps) composed of four proteins and pRNA exhibited RNase P activity, though they are catalytically less effective than R-5P composed of five proteins and pRNA. The present result revealed that the individual proteins contribute to RNase P activity in order of *Pho*Pop5 > *Pho*Rpp30 > *Pho*Rpp21 >> *Pho*Rpp29 > *Pho*Rpp38, though they are dispensable for RNase P activity *in vitro*. Interestingly, *Pho*Rpp38 makes little contribution to RNase P activity at a lower temperature. The amino acid sequence of *Pho*Rpp38 is highly homologous to that of *H. marismortui* ribosomal protein L7Ae, and therefore, it may function as ribosomal protein L7Ae in the *P. horikoshii* ribosome. In addition, it is known that the archaeal ribosomal protein L7Ae is a multifunctional protein, functioning in ribosome as well as RNP complexes (box C/D and box H/ACA) (*28*), which are involved in modification of rRNA as well as tRNA. It is thus speculated that the hyperthermophilic RNase P could employ the multifunctional RNA binding protein *Pho*Rpp38 for adaptation to a higher temperature.

The *in vitro* reconstitution of human RNase P indicated that two proteins Rpp21 and Rpp29 and H1 RNA are the minimal component of human RNase P (*8*). Furthermore, Rpp29 is known to be able to substitute for the *E. coli* C5 protein in reconstitution assays of M1 RNA activity (*8*, *29*). In addition, *in vitro* reconstitution using *P. furiosus* components provide the evidence that two proteins *Pfu*Pop5 and *Pfu*Rpp30 or *Pfu*Rpp21 and *Pfu*Rpp29 reconstituted an active RNase P activity in the presence of the intact RNA subunit (H.-Y. Tsai and V. Gopalan, personal communication). In the present analysis, the incubations of *Pho*Pop5 and *Pho*Rpp30 or *Pho*Rpp21 and *Pho*Rpp29 with pRNA produced no particles with RNase P activity. In addition, the contribution of *Pho*Rpp29 to RNase P activity was found to be lower than those of other three proteins *Pho*Pop5 and *Pho*Rpp30 or *Pho*Rpp21. These results are inconsistent with the results obtained for the *P. furiosus* and human RNase Ps. It was reported that the *B. subtilis* protein influences substrate recognition by RNase P (*26*), and that *E. coli* protein, but not the *B. subtilis* protein,

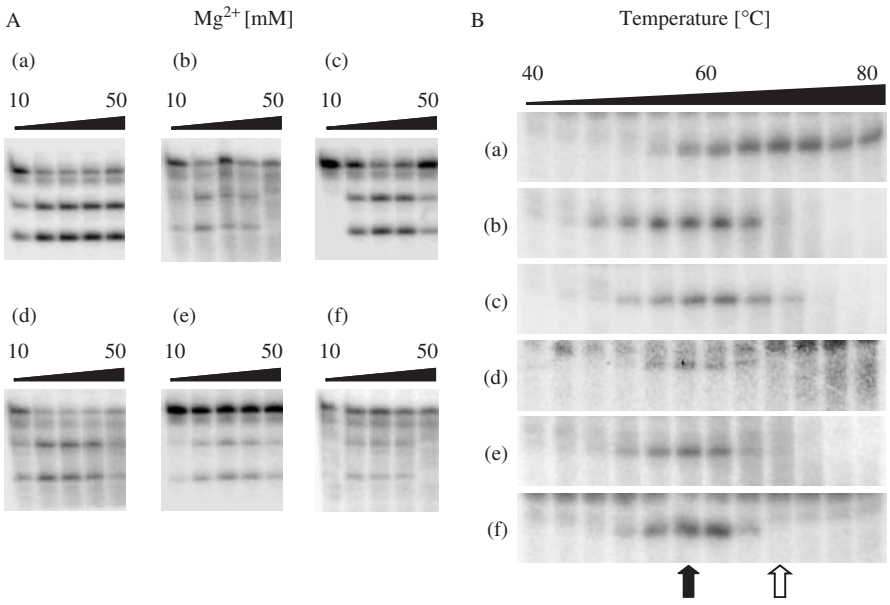

Fig. 3. **Characterization of the enzymatic activity of the *in vitro* reconstituted RNase P.** A: The RNase P activities of R-4Ps were characterized in terms of Mg^{2+} ions. The cleavage reactions were performed in the reaction buffer containing 10, 20, 30, 40, and 50 mM Mg^{2+}. The reaction times varied by composition of RNase P subunits, that is, 15 min (R-5P), 6 h (R-4P lacking *Pho*Rpp29 or *Pho*Rpp38), or 15 h (R-4P lacking *Pho*Pop5, *Pho*Rpp21, or *Pho*Rpp30). B: Optimal temperature for the RNase P activities of R-4Ps. The reactions were done at the indicated temperatures from 40°C to 80°C in the same manner as those described in Fig. 1. (a–f) indicate the reconstituted particles as given in Fig. 2A. Opened and closed arrows indicate optimal temperatures for R-5P and R-4Ps, respectively.

stabilizes the global structure of RNase P RNA, although both proteins influence dimmer formation of holoenzymes and pre-tRNA recognition (*27*). It is likely that homologous proteins may not play an equivalent function in RNase Ps from distinct organisms.

It is known that *B. subtilis* pRNA folds in three distinct conformations in folding pathway: unfold (U), intermediate (I), and native (N) (*30*, *31*). The I state was shown to be uniform under a variety of conditions and was therefore postulated to represent a well-defined thermodynamic state. Furthermore, the transition from the U state to the I state involves substantial secondary structure formation and results in a significant compaction of the RNA. The transition from the I state to the N state involves tertiary structure formation and requires the cooperative binding of at least three Mg^{2+} ions. In the current study, the four proteins and pRNA produced the particles R-4Ps with optimal temperature around at 55°C, and addition of the fifth proteins equally elevates the optimal temperature from 55°C to 70°C. This result suggests that pRNA interacted with four proteins would fold in a structure equivalent to the I state and addition of the fifth proteins would facilitate transition from the intermediate structure to a native structure corresponding to the N state of *B. subtilis* RNase P RNA. Further study on pRNAs in R-5P and R-4Ps will be required to address this assumption.

The present study reveals that all five particles R-4Ps exhibited RNase P activity, indicating that the *P. horikoshii* RNase P proteins are individually dispensable for RNase P activity *in vitro*. This result is not unexpected, because our present study indicated that the *P. horikoshii* pRNA, like eubacterial pRNAs, plays a crucial role in RNase P activity. Thus, site-directed mutagenesis of A40 and A41 in the *P. horikoshii* pRNA, which correspond to essential nucleotides A65 and A66 in *E. coli* pRNA, strongly reduced the RNase P activity (Terada *et al*. unpublished results). The present study reveals that *Pho*Pop5 and *Pho*Rpp30 play an important role in RNase P activity, while *Pho*Rpp29 and *Pho*Rpp38 make less contribution to the activity. The determination of crystal structures of the *P. horikoshii* RNase P proteins, together with the yeast two-hybrid analysis, has proposed a possible arrangement of the proteins in the *P. horikoshii* RNase P (*14*). In this model, a heterotetrameric structure composed of two proteins *Pho*Pop5 and *Pho*Rpp30 makes a core structure for assembly for other two proteins *Pho*Rpp21 and *Pho*Rpp29. The present result that shows an important role of *Pho*Pop5 and *Pho*Rpp30 in RNase P activity and less contribution of *Pho*Rpp29 and *Pho*Rpp38 to the activity seems to correlate with a spatial arrangement of the model. Thus, the proteins *Pho*Pop5 and *Pho*Rpp30 which are predicted to be core proteins are assigned as crucial proteins, while the protein *Pho*Rpp38 which has no protein partner is as a negligible protein. This finding led us to the speculation that a correct assembly of the individual proteins would be required for RNase P activity, then a protein complex thus formed might play an essential role in stabilization of a catalytic conformation of pRNA as an RNA chaperon.

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